bio-protocol Preprint

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RNA isolation, RT-PCR, and library preparation

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An abbreviated version of this protocol was published in Molecular Biology of the Cell in Sep 2021

The SON RNA splicing factor is required for intracellular trafficking structures that promote centriole assembly and ciliogenesis

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Detailed protocol

RT-PCR recipe and conditions

cDNA synthesis:
8 µl total of 1 µg of RNA
1 µl 50 µM Random Hexamer Mix (Invitrogen)
1 µl 10 mM ea dNTPs
65°C, 5 min
Ice, 5 min
4 µl H_2O
4 µl 5X FS Buffer (Invitrogen)
1 µl 0.1M DTT
1 µl SuperScript III (Invitrogen)
25°C, 5 min
50°C, 60 min
70°C, 15 min
Ice.
PCR:
Dilute cDNA 1:5 into H_2O
10.9 µl H_2O
4 µl cDNA
2 µl 10X Taq Buffer*
1.6 µl 25 mM $MgCl_2$
0.4 µl 20 µM Forward Primer**
0.4 µl 20 µM Reverse Primer**
0.2 µl 10 mM ea dNTPs
0.5 µl Taq DNA pol.

Cycle conditions:

1	95°C	0:30
2	95°C	0:10
3	57°C	0:20
4	72°C	0:20
5	back to step 2 (36 cycles for CEP131, 35 cycles for CNTROB, 30 cycles for Actin)	
6	72°C	7:00
7	4°C	hold

* 10X Taq Buffer:
500 mM KCl
100 mM Tris pH 8.5
0.1% Triton X-100

** Primer pairs:
ACTB-F: AGAGCTACGAGCTGCCTGAC; ACTB-R: AGCACTGTGTTGGCGTACAG;
CNTROB-4F: TGCAAGACTTGTCTCCATCTAGCTC; CNTROB-5R: TTGTCCAGTTGTTCAATCATGGTATCTTTC;
CNTROB-9F: AGAAGAGCCAGAGGGAAGCC; CNTROB-10R: TTGCCGTAGGCTGCTCTCC;
CEP131-4F: ACGGAGCCCACAGACTTCC; CEP131-6R: CGCAGTTGCCCACTGCTC;
CEP131-14F: TGGGGTCCGAGGTGAGC; CEP131-15R: GCTGGATGGTGGCCTCG

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Stemm-Wolf, A. and Pearson, C. (2022). RNA isolation, RT-PCR, and library preparation. Bio-protocol Preprint. bio-protocol.org/prep1805.
2. Stemm-Wolf, A. J., O'Toole, E. T., Sheridan, R. M., Morgan, J. T. and Pearson, C. G.(2021). The SON RNA splicing factor is required for intracellular trafficking structures that promote centriole assembly and ciliogenesis. Molecular Biology of the Cell 32(20). DOI: 10.1091/mbc.E21-06-0305